Alytes, 1993, **11** (1): 25-35.

Skin morphology in larval, paedomorphic and metamorphosed Alpine newts, *Triturus alpestris apuanus*

Franco ANDREONE*, Bruno DORE**,
Pasquale USAI** & Adriana PARANINFO**

* Museo regionale di Scienze naturali, Sezione di Zoologia,
via Giovanni Giolitti 36, 10123 Torino, Italy

** Dipartimento di Biologia animale, Università degli Studi di Torino,
via Accademia Albertina 17, 10123 Torino, Italy

Histological data on the skin of larval, paedomorphic and metamorphosed specimens of the Alpine newt, *Triturus alpestris apuanus*, have been analyzed and compared with natural history information. The skin of early larvae is composed of a few cell layers and contains Leydig cells, but lacks exo-epithelial dermal glands, which appear later during the premetamorphic stage. In metamorphosed newts the skin is typically multilayered and cornified, with abundant mucous and serous glands. The situation in paedomorphics, which are branchiate and live in water, is variable: in some cases (corresponding to immature "giant larvae"), the skin presents larval aspects (Leydig cells are present), while in others (usually sexually active and paedogenetic individuals) it is similar to that of metamorphosed newts. Thus metamorphosis in paedomorphic newts is not abandoned, but only delayed. The result is a mosaic of larval and metamorphosed characteristics, which can be related to the highly plastic ecology of these newts.

INTRODUCTION

The typical life cycle of urodeles is amphibious, with aquatic larvae and metamorphosed individuals living in a terrestrial habitat. Nevertheless, paedomorphism (sensu DUBOIS, 1987, or neoteny, sensu BREUIL, in press, understood as the retardation in the development of somatic and/or gonadal features or as the achievement of the sexual maturity while retaining larval-juvenile characters) often occurs. Paedomorphic newts have external gills, reach a greater size than larvae, and are totally aquatic. The causes of paedomorphism are not well known, although ecological constraints are often invoked (WILBUR & COLLINS, 1973). Among the European urodeles, the Alpine newt, *Triturus alpestris* (Laurenti, 1768), throughout its range and involving most subspecies, shows a high incidence of paedomorphism. Recently, as a part of a wider study of paedomorphism in Italian Alpine newt populations (ANDREONE & DORE, 1991), some preliminary hypotheses on the ecological basis of the phenomenon were formulated, moreover,

information has been gathered on variations in the histology of thyroid glands and gonads of paedomorphic and metamorphosed individuals (ANDREONE, DORE & USAI, 1991). In the present paper, as a complement, data on skin morphology during the life cycle are presented, taking into account that the amphibian integument witnesses the changes from an aquatic to a terrestrial habitat, acting as a medium for ionic exchange (DUELLMAN & TRUEB, 1986; LODI et al., in press).

MATERIAL AND METHODS

The population studied (which belongs to the subspecies *Triturus alpestris apuanus*), inhabits an artificial temporary pond located between the towns of Murazzano and Bossolasco (southern Piedmont, north-western Italy), at an altitude of about 700 m, with a surface area of about 100 m^2 and a maximum depth of 1.5 m. No aquatic vegetation (except *Chara* algae) is present, while other amphibians living there are the common frog (*Rana temporaria*) and the common toad (*Bufo bufo*). The climate of the area is Mediterranean (MENNELLA, 1967), with maximum rainfall in May and November. In this site a remarkable number of aquatic newts are found in the water throughout the year, even during non-reproductive periods.

Each month, from January 1988 to December 1989, several aquatic newts were caught by hand-netting (ANDREONE & DORE, 1992). The following categories were examined:

(1) Larvae, i.e. branchiate individuals with a total length less than 40 mm, having a brownish-greenish back, scattered darker spots, and a whitish belly (fig. 1). Sometimes two age cohorts of larvae may co-exist in the same pond, resulting from two egg depositions at different times during a year (ANDREONE & DORE, 1992).

(2) Metamorphosed, i.e. sexually mature newts. Such individuals court during the breeding season, when they display secondary sexual characters (SSC), such as lateral white stripes, extended dorso-caudal crests and swollen cloacae in males, and a swollen abdomen and turgid cloaca in females (fig. 2). Although the metamorphosed aquatic population is mainly represented by adults, juvenile specimens also are found; such individuals are smaller than sexually mature individuals, do not show SSC and, in some cases, have a light dorsal line.

(3) Paedomorphics (sensu lato), i.e. individuals with external gills (more or less developed), larger body size than larvae and with a yellow-orange belly. We refer to "neoteny" or "paedomorphism" according to KOLLMANN (1884a-c) and DUBOIS (1987): "paedomorphic" to indicate branchiate newts without SSC and lacking the courtship behaviour, corresponding to the "partial neotenic" (sensu BREUIL, in press) (fig. 3), and "paedogenetic" (= "total neotenic") to indicate the branchiate newts displaying courtship behaviours and SSC (fig. 4).

After capture, newts were anaesthetized by immersion in a 0.5 % MS 222 Sandoz solution. Some individuals were dissected to verify their gonadal status, and a portion of their dorsal and ventral skin was cut off, fixed in cold buffered formalin, dehydrated, and infiltrated overnight with a glycol metacrylate monomer ("Technovit 7100" Kulzer) in a

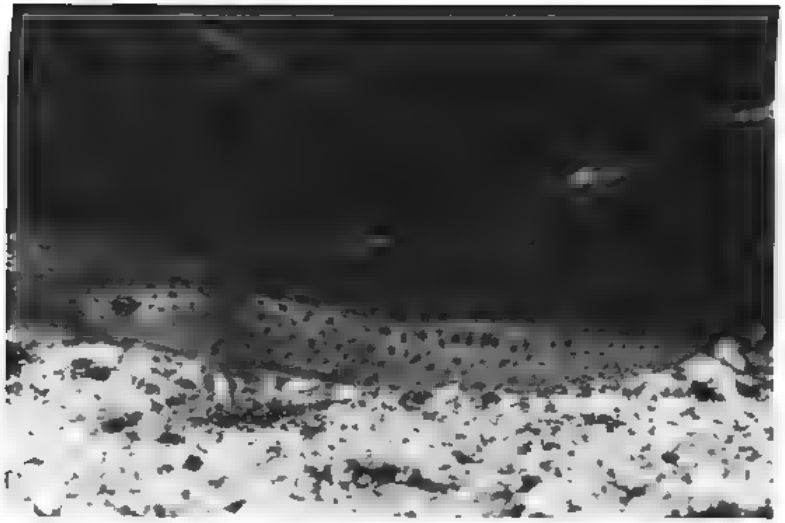

Fig. 1. – Larva of Alpine newt (*Triturus alpestris apuanus*), characterized by external gills, continuous caudal crests, and a light brownish scattered back.

Fig. 2. – Metamorphosed male of Alpine newt during the breeding season, with well developed secondary sexual characters (SSC), e.g. dorsal and caudal crests, white lateral stripe, blue back coloration and swollen cloaca.

Fig. 3. – Paedomorphic (partial neotenic and sexually immature giant larva) Alpine newt, characterized by a general larval aspect, undeveloped gonads and by the absence of evident SSC.

Fig. 4. – Paedogenetic (totally neotenic) adult male of Alpine newt, with external gills and well developed secondary sexual characters.

ice bath. Polymerization was carried out at about 6-10°C to preserve enzymatic activities (Dore & Usai, 1986; Andreone & Dore, 1992). Skin sections of 2 µm were stained with acid fuchsin-toluidine blue (Dougherty, 1981), and with Burstone direct coupling method of substituted naphtols contrasted with methyl green for Alkaline Phosphatase (APH) activity (Mazzi, 1977; Loyda, Gossrau & Schleber, 1979). APH activity may be correlated with transcutaneous ionic transport (see Lodi et al., in press). Controls were carried out in the absence of substratum.

Results

Larvae

The larval skin is composed of a few cell layers (three or four) lying on a dense layer of dermis ("basement lamella", Fox, 1977). In all specimens examined the epithelial basal cells have an elongated nucleus, are irregular in shape, and penetrate with their apical part among the overhanging Leydig cells. The latter cells, well known in larvae and neotenic urodeles (see Hay, 1961; Raffaelli, 1989) have a poorly known function, but they probably are glandular (Fox, 1988) and secrete mucus into subsurface extracellular compartments of the epidermis (Duellman & Trueb, 1986). Leydig cells are large and rounded, with a clear cytoplasm, PAS-positive granules and prominent nucleus, and they occupy the entire epidermal thickness and press against the common epithelial cells with their convex surface. We did not observe a general uniformity in the morphology and disposition of cells in the skin of different larvae. However, in early larvae the Leydig cells are disposed in two layers (basal and sub-apical) and contain abundant PAS-positive granules, while in older larvae they form only one layer and have a scarce granulation. The skin surface is not cornified, and is composed of one or two layers of flattened epithelial cells, while isolated supporting cells are interposed among the underlying Leydig cells. Neuromasts of the lateral line are constantly present. In early larvae the dermis is thin and usually not invaded by glandular (serous and mucous) elements. In some large premetamorphic larvae or in larvae becoming paedomorphic (see later), precocious gland buds begin to differentiate (fig. 6). Low APH activity is seen, and – when visible – it is localized in the most external epithelial cells, whereas it is totally lacking in the dermis, except in the blood vessels of the subcutaneous layer.

Metamorphosed specimens

The epidermis of metamorphosed Alpine newts (either sexually mature or newly metamorphosed) is similar to that described for *Triturus carnifex* (Lodi, 1968; Lodi & Bani, 1971), being about four to six cells thick. Migration of deep cells occurs towards the surface. Cells of the basal or germinative layer are large and irregularly shaped, generally columnar or cuboidal, and arrayed in a low palisade. Mitoses are often visible in the germinative layer, and melanophores are commoner in the dorsal dermis than elsewhere, but some scattered melanophores may be found in the dorsal epidermis (Duellman & Trueb, 1986). Flask cells (involved in the sodium transport; see Fox, 1986a-b; Zaccone

Fig. 5. – Histological structure of the skin in an Alpine newt larva. Two layers of Leydig cells with PAS-positive granules are evident, together with plate superficial cells. Toluidine blue coloration. The bar corresponds to 10 μm.

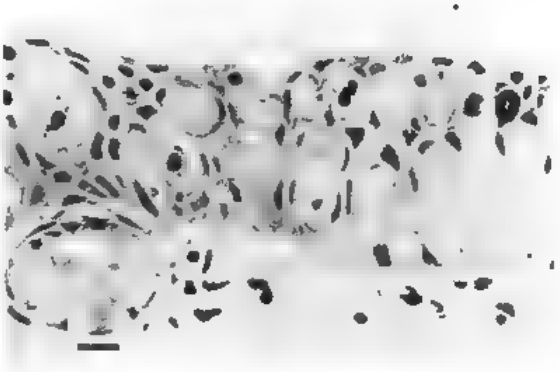

Fig. 6. – Late larval skin, with Leydig cells, with a dermal gland bud. Toluidine blue coloration. The bar corresponds to 10 μm.

Fig. 7. – Histological structure of the skin in a metamorphosed Alpine newt. Great exoepithelial glands are visible, together with several cell layers and a cornified surface. Toluidine blue coloration. The bar corresponds to 10 μm.

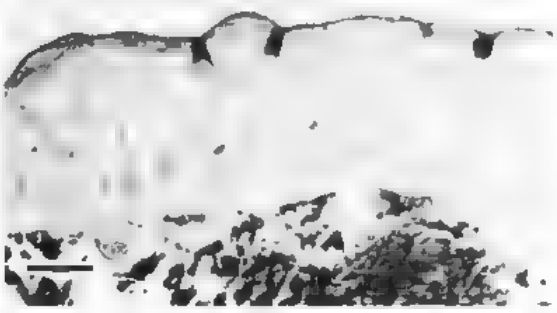

Fig. 8. – Metamorphosed skin, with flask cells shown by means of APH and Burstone's method of substituted naphtols contrasted with methyl green. The bar corresponds to 10 μm.

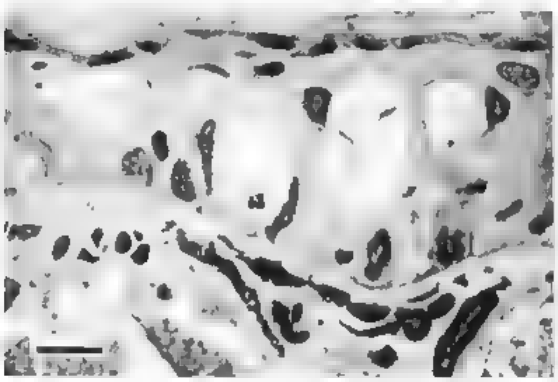

Fig. 9. – Structure of the skin in a paedomorphic immature Alpine newt. Empty Leydig cells (disposed in a single layer) with scarce PAS-positive granules are visible. Mitoses are evident in the cells among Leydig cells. Toluidine blue coloration. The bar corresponds to 10 μm.

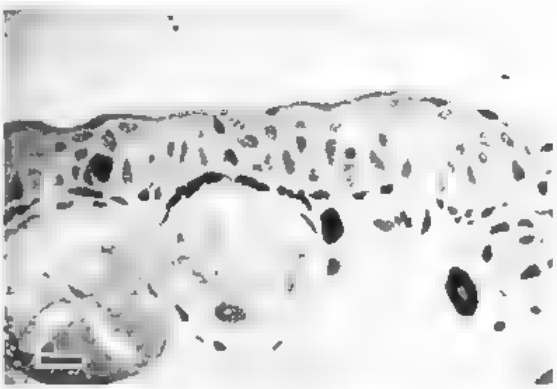

Fig. 10. – Skin of a paedogenetic Alpine newt the structure and morphology – even with a reduced superficial cornification – is similar to the metamorphosed one. Toluidine blue coloration. The bar corresponds to 10 μm.

et al., 1986) are distributed more or less regularly in the subcorneous layer (fig. 7). The skin surface is cornified, with protruded warts, two or three flattened cells thick Highly developed mucous and serous glands are always evident in the spongy thick dermis just below the epithelium either in the ventral or in the dorsal skin. In metamorphosed newts, APH activity is similar in the dorsal and in the ventral skin, varying from low to moderate in the cells of corneous and subcorneous layers, but lacking in the deepest layers. Flask cells usually show an intense APH activity (fig. 8) in their neck region. In the dermis, a high APH activity is evident, particularly in the blood vessels and around and inside the glands.

Paedomorphic newts

In paedomorphic newts, skin morphology is rather variable and not easily generalized. In animals showing non-functional gonads and without evident SSC, histological organization is roughly intermediate between that of larval and metamorphosed skin The superficial cells are relatively loose (somewhere separated by empty spaces), neuromasts can be seen (even if rarer than in larvae), large exo-epithelial glands are present, and Leydig cells (Fox, 1988) are not so orderly as in larvae, have a lighter cytoplasm, scarce granules. In these animals mitoses can be observed in cells interposed among Leydig cells (fig. 9).

In some paedomorphic specimens (Gabrion & Sentein, 1986), a mosaic situation can be noticed: the Leydig cells are still present and abundant in some areas, while in others they are absent. In other branchiate newts (sexually mature and paedogenetic), the skin is highly similar to that of the metamorphosed ones, although with a lower surface cornification (fig. 10). It is sometimes possible to observe empty spaces inside the epithelium, probably derived from the degeneration of Leydig cells. APH activity in the more superficial layers is usually scarce or even absent, whereas in the dermis it is localized in the portion just below the basal layer of the epithelium and more evident around the glands than elsewhere. Flask cells are present in a few paedogenetic specimens and show a high APH activity in the portion superficial to the nucleus.

Discussion

Analysis of the organization of skin of Alpine newts throughout the life cycle discloses high variability from larvae to metamorphosed specimens. The skin of paedomorphic specimens has a structure which varies from larval-like to metamorphosed-like organization, sometimes the skin shows both larval characteristics (e g Leydig cells, neuromasts) and metamorphosed characteristics (e g. dermic glands, horny surface). This intermediate situation is also evident in the activity of APH, as has been stressed by Lodi et al. (in press). These data mainly agree with Gabrion & Sentein (1976), who observed that Leydig cells vary in number and shape from totally larval to late paedomorphic palmate newts (*Triturus helveticus*), in which the skin closely resembles that of meta-

morphosed individuals. In late paedogenetic Alpine newts, the skin rearrangement extends also to the empty spaces left by the disappearance of the Leydig cells. The mitoses observed in the intermediate epithelium can be interpreted as part of a regenerative process.

The variability reported suggests the persistence (and not the arrest) of metamorphic phenomena even in paedomorphic Alpine newts, and stresses the difficulty in categorizing branchiate newts. In fact, by external examination it is possible to observe sexually inactive paedomorphic individuals which resemble larvae, and others (paedogenetic) which exhibit a typical mating coloration (more visible in males), and are sexually active, behaving during the courtship like the typical metamorphosed adults (ANDREONE, 1990; BOVERO, 1991). Natural history observations confirm that gill development and length vary among paedomorphic individuals of the very same population and that these may – at a certain moment of their life – metamorphose. Thus, it is not possible to consider the paedomorphics as a whole and uniform category, because they are part of a continuum, from larval to metamorphosed newts. In this sense it is worth reaffirming, in contrast to MOLA & BERTOLANI (1981), that the presence of Leydig cells is not a constant neotenic character. Perhaps these authors analyzed the skin of larval-like paedomorphic newts ("giant overwintered larvae") and not that of the typical paedogenetic newts. The correlation between morphology and function of the skin has been pointed out by LODI et al. (in press): a typical larval organization (with Leydig cells) is characterized by the absence of active sodium transport, that is evident when the epidermis acquires, at least in part, the metamorphosed characteristics.

All these considerations indicate how the newt life cycle (and more particularly that of *Triturus alpestris*) cannot be rigidly schematized, but that plasticity in life history characteristics is itself adaptive. In fact, the highly aquatic Alpine newts synchronize their permanence in the water both to altitude differences (and thus to the duration of their breeding period) and to desiccation-rain alternation in unpredictable habitats (ANDREONE et al., in press). An outline of the life history of Alpine newts is represented in fig. 11 – to interpret the observations on skin morphology and on external features (modified from VERRELL, 1985, DŽUKIĆ et al., 1991, and ANDREONE et al., in press). Typically, newts spend the first part of their existence as aquatic larvae; thereafter metamorphosis induces some drastic changes (e.g. in the skin structure) as the newts become terrestrial. Growth until sexual maturity occurs on land (particularly when the breeding site is temporary), but juveniles may return to water for non-reproductive reasons, as has been reported for other species by VERRELL (1985) and ANDREONE & GIACOMA (1989). Nevertheless an alternative strategy is observed in more stable habitats: adulthood is reached through a direct aquatic development, and larvae may overwinter in the water (especially those hatched in autumn, see ANDREONE & DORE, 1992), becoming paedomorphic juveniles. Later they may become paedogenetic, thus displaying SSC and courtship patterns. Throughout this aquatic development, skin morphology modifies, although the changes are not so drastic as in the transformation from water to land in the typical life cycle. For this reason a mosaic situation is seen, and neotenic individuals may present both larval and metamorphosed characters. Nevertheless metamorphosis may occur any time in the aquatic development. Thus, metamorphosed individuals found in the water may result either after growth in a terrestrial habitat, or by transforming at various stages of their aquatic life, possibly on

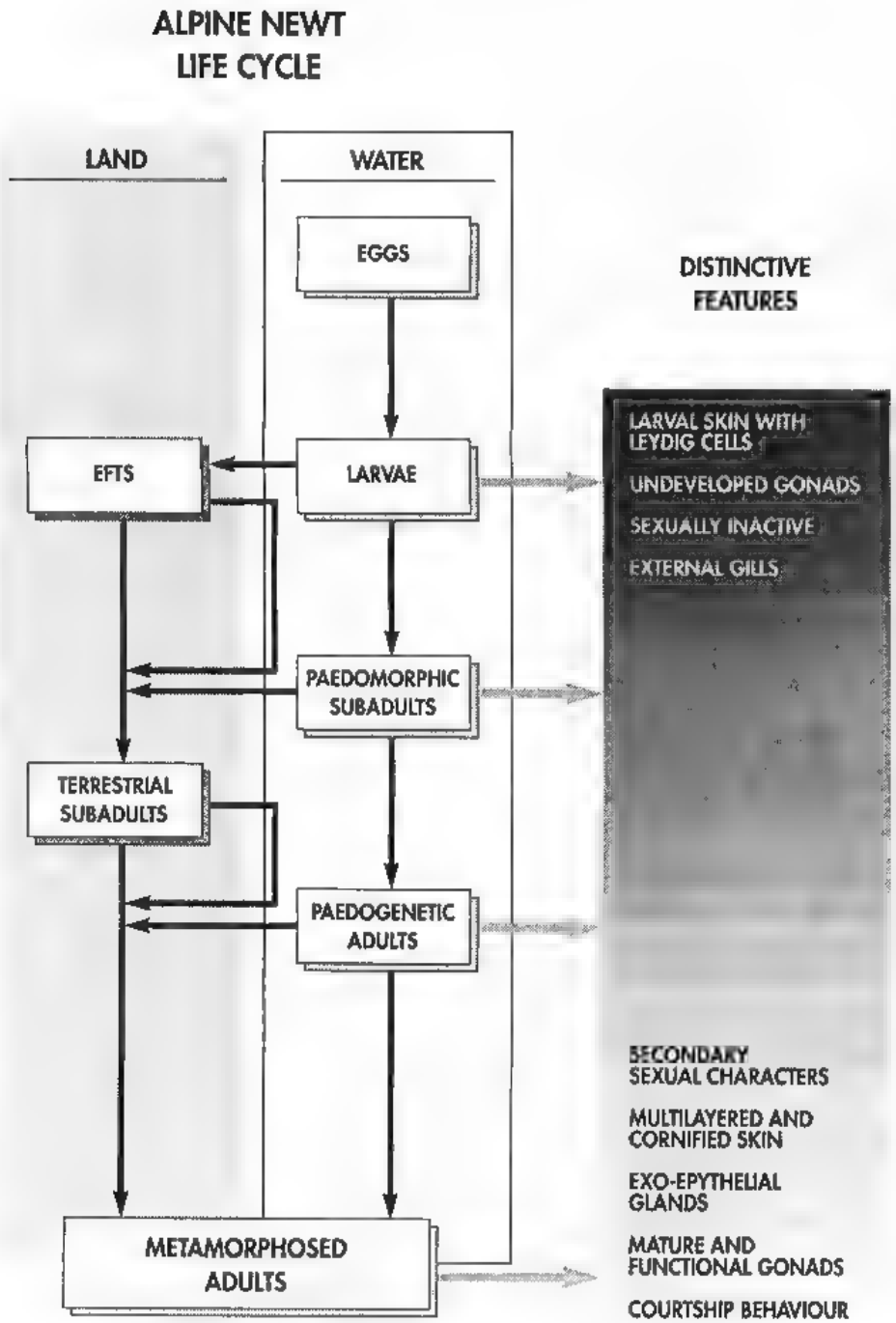

Fig. 11. Outline of the life cycle in *Triturus alpestris apuanus*. The left part summarizes the passages between water and land throughout growth and metamorphosis. The black arrows indicate habitat changes, but do not suggest any preference, depending on the characteristics and constraints of each individual and population. The right part summarizes some typical features of the extreme categories of aquatic newts, from an early larval stage to a final paedogenetic-metamorphosed phase. Grey arrows indicate the correspondence of phases and biological features. Between these two extremes several intergrades are present. The distinctive features of metamorphosed newts, excluding the characteristics related with sexual activity, are common both to adults and to juveniles.

the occasion of pond desiccation or of water pollution (see ANDREONE & DORE, 1991). The reasons for this plasticity should be sought in adaptive explanations: paedomorphism – as remarked by BREUIL (in press) – may lead to earlier sexual maturity, life in a more stable aquatic habitat, or better utilization of trophic resources (KALEZIĆ, DŽUKIĆ & TVRTKOVIĆ, 1990).

RÉSUMÉ

L'histologie de la peau du triton alpestre (*Triturus alpestris apuanus*) a été analysée et comparée avec des données concernant l'histoire naturelle de l'espèce. La peau des jeunes larves est composée d'un nombre réduit de couches cellulaires et contient des cellules de Leydig, mais pas de glandes extra-épithéliales; celles-ci deviennent visibles plus tard, avant la métamorphose. Chez les tritons métamorphosés elle est typiquement pluri-stratifiée et cornifiée, avec beaucoup de glandes muqueuses et séreuses. L'organisation épithéliale des individus pédomorphiques, qui ont des branchies et vivent dans l'eau, est assez variable: chez certains exemplaires (correspondant aux larves géantes immatures), la peau présente des caractéristiques typiquement larvaires (par exemple les cellules de Leydig sont encore présentes), tandis que chez d'autres (habituellement sexuellement actifs bien que pédogénétiques) elle est très similaire à celle des tritons métamorphosés. Ainsi, chez les tritons pédomorphiques, la métamorphose n'est pas abolie, mais seulement retardée. Il en résulte une mosaïque de caractéristiques larvaires et post-larvaires. Ces caractéristiques sont en relation avec l'écologie plastique du triton alpestre.

ACKNOWLEDGEMENTS

Thanks are due to M. BREUIL for bibliographic support and for the exchange of information about neoteny, and to two anonymous referees for critical advice. This work was supported by grants from Ministero della Pubblica Istruzione and Ministero dell'Università e della Ricerca scientifica e tecnologica (funds 40 % and 60 %).

LITERATURE CITED

ANDREONE, F., 1990. *Variabilità morfologica e riproduttiva in popolazioni di* Triturus alpestris *(Laurenti, 1768) (Amphibia Salamandridae)*. Doctoral research thesis, University of Bologna.

ANDREONE, F. & DORE, B., 1991. – New data on paedomorphism in Italian populations of the Alpine newt, *Triturus alpestris* (Laurenti, 1768) (Caudata Salamandridae). *Herpetozoa*, **4** (3/4): 149-156.

----- 1992. Adaptation of the reproductive cycle in *Triturus alpestris apuanus* to an unpredictable habitat. *Amphibia-Reptilia*, **13**: 251-261

ANDREONE, F., DORE, B. & USAI, P., 1991. — Histological and ecological aspects of neoteny in *Triturus alpestris apuanus* (Bonaparte). *In* G. GHIARA et al. (eds.), *Symposium on the evolution of terrestrial vertebrates*, U.Z.I. Selected Monographs and Symposia, Modena, Mucchi Editore, vol. 4: 413-420.

ANDREONE, F. & GIACOMA, C., 1989. — Breeding dynamics of *Triturus carnifex* at a pond in north-western Italy (Amphibia, Urodela, Salamandridae). *Holarct. Ecol.*, **12** (3): 219-223.

ANDREONE, F., GIACOMA, C., CAVALLOTTO, L. & FRANCILLON-VIEILLOT, H., in press. — Le cycle reproducteur de *Triturus alpestris*: influence des facteurs externes. *Actes du Colloque d'Ecologie et de Biogéographie Alpines.*

BOVERO, S., 1991. — *Analisi del comportamento riproduttivo in esemplari sintopici — metamorfosati e pedogenetici — di* Triturus alpestris apuanus *(Amphibia Salamandridae)*. Degree thesis, University of Turin.

BREUIL, M., in press. — La neoténie dans le genre *Triturus*: mythes et réalités. *Bull. Soc. herp. Fr.*

DORE, B. & USAI, P., 1986. — Glycol methacrylate embedding for enzyme preservation. *Bas. appl. Histochem.*, **30** (suppl.): 82.

DOUGHERTY, W., 1981. — Preparation of semi-thin sections of tissues embedded in water soluble methacrylate for light microscopy. *In*: G. CLARK (ed.), *Staining procedures*, Baltimore & London, William & Wilkins, Biological stain commission.

DUBOIS, A., 1987. — Neoteny and associated terms. *Alytes*, **4** (4): 122-130.

DUELLMAN, W. E. & TRUEB, L., 1986. — *The biology of amphibians*. New York, McGraw Hill.

DŽUKIĆ, G., KALEZIĆ, M. L., TVRTKOVIĆ, N. & DKPRPVOC, A., 1991. — An overview of the occurrence of paedomorphosis in Yugoslav newt (*Triturus*, Salamandridae) populations. *BHS Bull.*, **34**: 16-22.

FOX, H., 1977. — The anuran tadpole skin: changes occurring in it during metamorphosis and some comparisons with that of the adult. *In*: R. I. C. SPEARMAN (ed.), *Comparative biology of the skin*, Symp. zool. Soc. London, London & New York, vol. 39: 269-289.

----- 1986a. — Changes in amphibian skin during larval development and metamorphosis. *In*: G. NALLS & M. BOWNES (eds.), *Metamorphosis*. Symp. brit. Soc. dev. Biol., Oxford Univ. Press, vol. 8: 59-87.

----- 1986b. — The skin of Amphibia. *In*: J. BEREITER-HAHN, A. G. MATOLTSY & K. S. RICHARDS (eds.), *Biology of the integument*, vol. II, *Vertebrates*, Heidelberg, Springer: 78-135.

----- 1988. — *Riesenzellen*, goblet cells, Leydig cells and the large cells of *Xenopus*, in the amphibian larval epidermis: fine structure and a consideration of their homology. *J. submicrosc. Cytol. Pathol.*, **20** (2): 437-451.

GABRION, J. & SENTEIN, P., 1976. — Structure histologique de la peau et phénomènes de dégénérescence chez *Triturus helveticus* Raz. au cours de la métamorphose. *Bull. Soc. zool. Fr.*, **101**, suppl. 5: 33-39.

HAY, E. D., 1961. — Fine structure of an unusual intracellular supporting network in the Leydig cells of *Amblystoma* epidermis. *J. Biophys. Biochem. Cytol.*, **10**: 457-463.

KALEZIĆ, M. L., DŽUKIĆ, G. & TVRTKOVIĆ, N., 1990. — Newts (*Triturus*, Salamandridae, Urodela) of the Bukovica and Ravni Kotari regions (Yugoslavia). *Spixiana*, **13** (3): 329-338.

KOLLMANN, J., 1884a. — Das Ueberwintern von europäischen Frosch und Tritonen Larven und die Umwandlung des mexikanischen Axolotl. *Verh. Naturf. Ges. Basel*, (1883): 387-398.

----- 1884b. — L'hivernage des larves de grenouilles européennes et de tritons. La métamorphose de l'axolotl mexicain. *C. r. Assoc. fr. Avanc. Sci.*, (1883), **12**: 567-570, 570-571 (LATASTE's remarks).

----- 1884c. — L'hivernage des larves de grenouilles et de tritons d'Europe et la métamorphose de l'axolotl du Mexique. *Rec. Zool. Suisse*, **1**: 75-89.

LODI, G., 1968. — Il tegumento del tritone crestato in condizioni sperimentali diverse. *Boll. Zool.*, **35**: 415.

LODI, G., ANDREONE, F., DORE, B., PARANINFO, A., USAI, P. & BICIOTTI, M., in press. — Active sodium transport and morpho-functional organization in the skin of the Alpine newt, *Triturus alpestris*, during the life cycle. *Boll. Zool.*

LODI, G. & BANI, G., 1971. — Microscopic, submicroscopic and histoenzymologic features of the epidermis of the normal and hypophysectomized crested newt. *Boll. Zool.*, **38**: 111-125.

LOYDA, Z., GOSSRAU, R. & SCHLEBER, T. H., 1979. — *Enzyme histochemistry. A laboratory manual.* Springer-Verlag.

MAZZI, V., 1977. – *Manuale di tecniche istologiche ed istochimiche*. Padova, Piccin.

MENNELLA, C., 1967. – *Il clima d'Italia nelle sue caratteristiche e varietà e quale fattore dinamico del paesaggio*. Napoli, Edart.

MOLA, L. & BERTOLANI, R., 1981. – Osservazioni istologiche e istochimiche sul differenziamento della cute in esemplari metamorfosati e non di *Triturus alpestris apuanus* (Bonap.) (Amphibia Urodela). *Arch. ital. Anat. Embriol.*, **86** (3): 195-207.

RAFFAELLI, J., 1989. – Etat des données sur quelques traits de la biologie des Amphibiens Urodèles. *Bull. Soc. Herp. Fr.*, **48**: 1-21.

VERRELL, P. A., 1985. – Return to water by juvenile amphibians at a pond in southern England. *Amphibia-Reptilia*, **6**: 93-96.

WILBUR, H. M. & COLLINS, J. P., 1973. – Ecological aspects of amphibian metamorphosis. *Science*, **182**: 1305-1314.

ZACCONE, G., FASULO, S., LO CASCIO, P. & LICATA, A., 1986. – Enzyme cytochemical and immunocytochemical studies of flask cells in the amphibian epidermis. *Histocemistry*, **84**: 5-9.

Corresponding editor: Milos KALEZIĆ.